uOttawa Inter-Lab Study

Release

1. Work was preformed by Martin Hanzel.
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3. Data collected September 4, 2014.
4. All contributors consent to inclusion of data.

Protocol

1. Competent *Mach 1* E. coli cells were transformed with DNA taken from the iGEM 2014 Distribution and assembled using biobrick assembly, where applicable. Applicable constructs were assembled by inserting the downstream DNA fragment into a plasmid carrying the upstream fragment. Positive colonies were grown in liquid LB overnight and screened on a flow cytometer for GFP expression, then re-plated on LB plates with the appropriate antibiotic. Cells were grown up for 8 hours in liquid LB media in a shaking incubator at 37 C and 200 RPM, then measured in a flow cytometer.
2. Instrument used was a Beckman Coulter CyAn configured with a 488 nm laser and FITC filters.
3. Protocol used for flow cytometry: cells suspended in 50 mM sodium citrate at OD_{600} = 0.03. Cells in suspension were inoculated into a 96-well plate at OD = 0.03 and multiple measurements were taken for the same constructs and the same cell lines. Flow cytometry was run with a voltage of 450 V on the side-scatter detector and 600 on the FITC detector.
4. Cells were preliminarily screened for greater GFP expression than an untransformed *Mach 1* sample of cells.
5. Controls included sterile water and untransformed *Mach 1* cells as negative controls, and Beckman Coulter *Flow-Set Pro Fluorospheres* as a positive control and reference.
6. We measured cell size, cell complexity, and GFP expression quantitatively. Other quantities, such as OD, were not measured precisely but rather served as a sample or guideline in our protocols.
7. The flow cytometry session took about an hour to set up and complete.
8. uOttawa iGEM pays a flat rate to use the flow cytometry facilities, and many of our reagents are prepared in-house. Therefore, a precise estimation of the cost to acquire this data is not available. However, many of the methods that we use are many times cheaper than standard methods found in literature.
9. Our flow cytometry facilities are very high-throughput and are capable of handling hundreds of thousands of events for every sample. We use a computer-controlled robot to analyse 96-well plates, further increasing the rate at which we can collect data. The largest restriction that we have faced is

that the small size of E. coli makes it difficult to differentiate between cells and random noise, thus producing very large files which our computers struggle to analyze quickly. A 96-well plate may produce an output data file that is greater than 500 MB in size. At these sizes, each well may take upwards of 30 seconds to identify and manipulate.

Measured Quantities

Optical density: measurements precise to 3 decimal places. Units are in % absorbance, relative to a control. Measurements range from 0 to 1. Measurements are less precise at values below 0.050. The instrument is calibrated against a control before every run. These answers were determined from past experience and interviews with other lab members.

Fluorescence: measurements precise, after analysis using a variety of software packages, to 2 decimal places, though fluorescence values for individual events are subject to much error and have no significance on their own. Units are in arbitrary units, as the sensitivity of the instrument may be adjusted depending on the experiment. Relative units are given as a fraction of fluorescence of standardized fluorescent beads. Fluorescence measurements range, in this experiment, from 0 to 189.71, although the machine is capable of measuring up to 10^3. Measurements are not reliable with cells smaller than *E. coli* due to autofluorescence of the medium and noise, however, fluorescence measurements are quite precise throughout the entire spectrum, provided that the instrument is set up correctly. The instrument is calibrated against a control before every run. These answers were determined from past experience, interviews with other users, and individual flow cytometry training.

Measurements

Red rows indicate outliers. These samples had arithmetic means that differed from the others by at least a factor of 2.

Mean fraction refers to the arithmetic mean divided by the mean of the +ve control.

Assembled construct containing the J23101 promoter: 2 cell lines of 5 samples

Sample	Arithmetic mean	Standard dev.	Mean fraction
C01	133.94	95.92	0.706025
C02	140.52	104.33	0.740710
C03	149.60	123.73	0.788572
C04	54.23	61.76	0.285857
C05	120.44	94.67	0.634864
D01	167.06	162.8	0.880607
D02	140.30	83.42	0.739550
D03	65.43	84.1	0.344895
D04	155.18	138.13	0.817985
D05	60.02	79.04	0.316378

Assembled construct containing the J23115 promoter: 5 samples

J23115 was used as distributed.

Sample	Arithmetic mean	Standard dev.	Mean fraction
E01	3.89	6.56	0.020505
E02	4.32	5.68	0.022772
E03	3.51	10.93	0.018502
E04	4.94	13.48	0.026040
E05	1.99	2.29	0.010490

Pre-built GFP construct I20260: 9 samples

Sample	Arithmetic mean	Standard dev.	Mean fraction
F01	17.83	15.02	0.093986
F02	19.36	15.86	0.102050
F03	17.62	12.78	0.092879
F04	17.67	11.33	0.093142
F05	18.99	12.26	0.100100
F06	18.78	12.98	0.098993
F07	15.68	12.70	0.082652
F08	17.64	11.83	0.092984
F09	15.93	11.58	0.083970

Controls

Sample	Arithmetic mean	Standard dev.	Mean fraction
Positive	189.71	10.80	1
Negative 1	0.38	0.19	0.002003

Means and standard deviations listed in the above tables describe the variation between individual cells.

Totals

Outliers were not included.

Construct	Arithmetic mean	Standard dev.	Mean fraction
J23101	143.8629	15.11104	0.75833
J23115	3.73	1.108355	0.019662
I20260	17.72222	1.266035	0.093417

Discussion

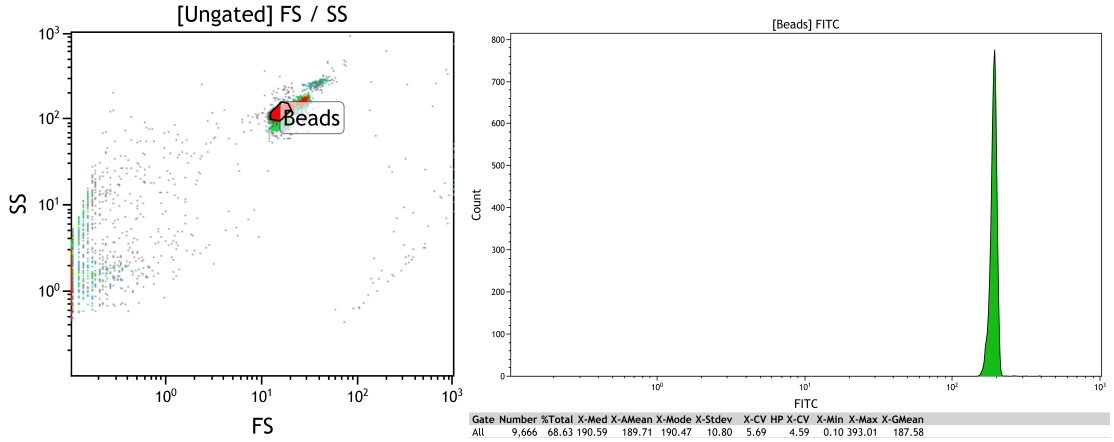
Given that the assembled construct with J23101 and I20260 share the same sequence, they should theoretically have identical expression levels. However, as I20260 was distributed in the medium-copy pSB3K3 vector, and J23101 in the high-copy pSB1C3 vector, it makes sense that I20260 would show lower expression.

Though some standard deviations may seem high, this is a side-effect of the flow cytometry process, as there exist negative and autofluorescent events in each sample that cannot reliably be gated out. In the section **Cell Variation**, plots of select samples are included.

Cell Variation

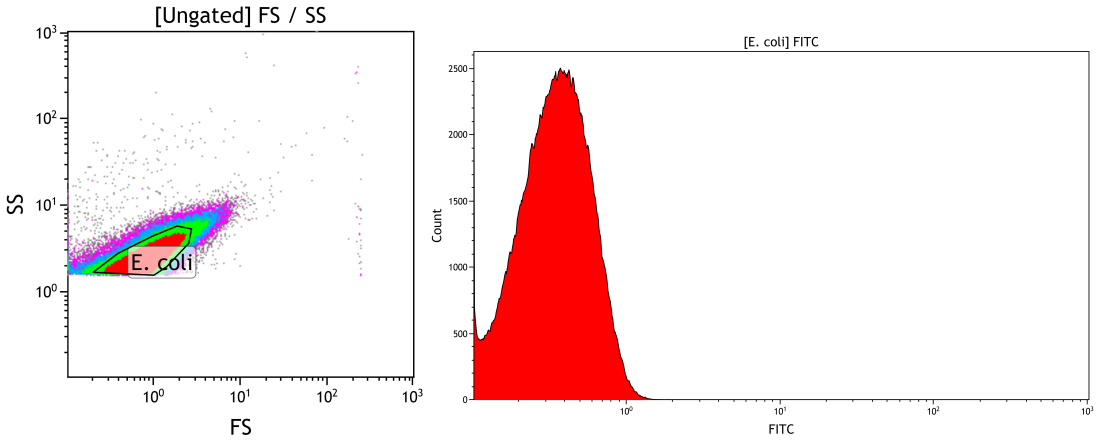
With flow cytometry, it is possible to measure the fluorescence of tens of thousands of cells individually in a short amount of time. Here, various plots and histograms are presented to show the range of fluorescence values for select samples.

Positive control



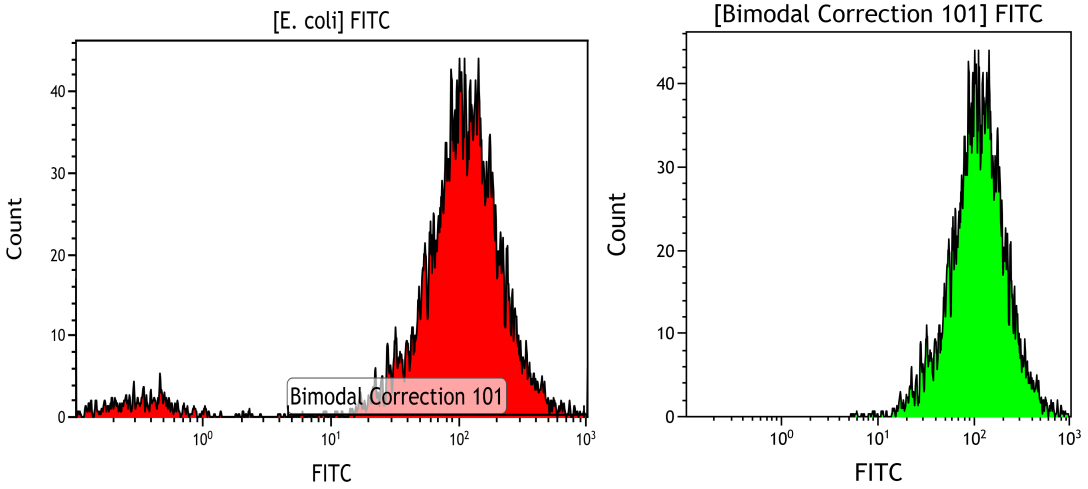
Left: side-scatter vs. forward-scatter analysis of fluorescent beads used as a positive control. The red area indicates a region that may be gated in order to obtain relevant results. **Right**: histogram of fluorescence for the positive control. Note the well-defined peak at just above 10^2 on the X axis.

Negative control



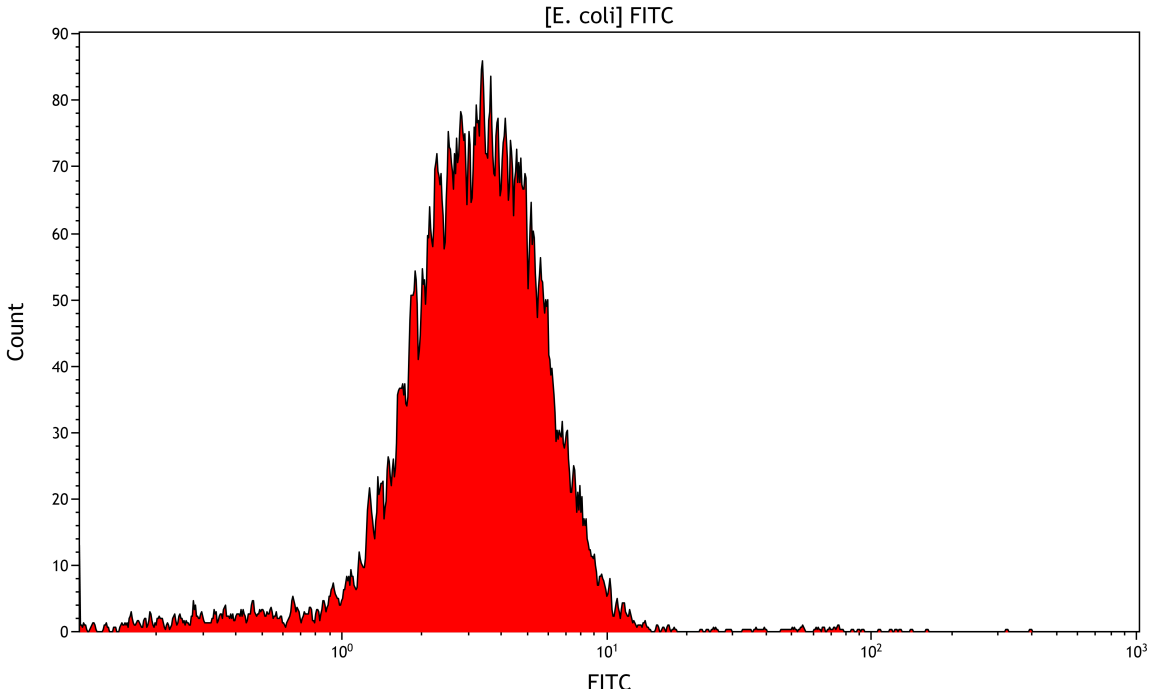
Left: side-scatter vs. forward-scatter of negative *E. coli* cells. The red region has been gated. Notice the sharp cutoff at just above 10^0 on the Y axis. This has been configured deliberately to reduce noise and keep the event count low for faster computation. Below the cutoff, cells are nearly indistinguishable from debris and noise. **Right**: histogram of fluorescence for negative *E. coli*.

Sample C01



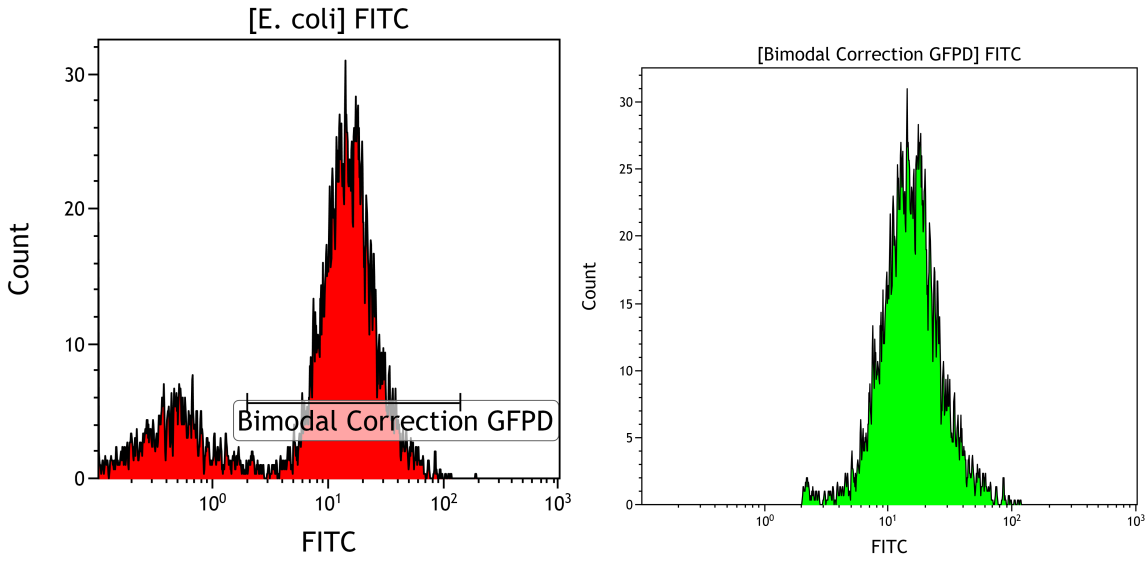
Left: histogram of fluorescence for sample C01. A shallow hump is visible where the negative control should be. This is a result of rapidly growing cells in media without antibiotic selection, so some cells eventually lose the plasmid containing GFP. These appear on the left as negatives. **Right:** Same sample with a correction applied to eliminate negative cells. This correction was used to compute the data given in the **Measurements** section.

Sample E1



Histogram of fluorescence for sample E1. It is clearly visible that there is noise to the left and right of the peak, which causes the standard deviation to be higher than expected. The E samples did not exhibit a strong peak at the left, and so a correction for negative cells was not applied. Further, the peaks for this construct and those of the negative control overlap slightly, so applying a correction would actually remove some positive events.

Sample F4



Left: histogram of fluorescence for sample F4. Again, negative cells may be seen. **Right**: same histogram after applying a correction. The range of events that were considered can be seen on the left figure.